Pergamon

Tetrahedron 56 (2000) 9985–9989

TETRAHEDRON

Cardiopetalolactone: A Novel Styryllactone from *Goniothalamus cardiopetalus*

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Received 30 June 2000; revised 19 September 2000; accepted 5 October 2000

Abstract—A new styryllactone namely cardiopetalolactone was isolated and characterized from the stem bark extract of *Goniothalamus cardiopetalus* (Annonaceae) together with two known lactones altholactone and goniopypyrone. The structure and stereochemistry of the new compound was determined on the basis of 2D NMR experiments, NOE difference spectroscopy and the Mosher ester technique.

Introduction

The genus *Goniothalamus* (Annonaceae) comprises 115 species of shrubs and trees growing in Asia and many of them are used in the folk medicine of several countries.[1] Phytochemical studies on many *Goniothalamus* species such as *G. amyun*,[2] *G. gigantius*,[3–7] *G. gifithi*,[8] *G. arvensis*,[9–10] and *G. leiocarpus*[11] have resulted in the isolation and characterization of two major classes of bioactive compounds including Annonaceous acetogenins and styryllactones. Both categories of these compounds possess complex stereochemistry and appear in different stereoisomeric forms with varying levels of cytotoxicity towards several human tumor cell lines. More than twenty styryllactones are known of which many are stereoisomers. *G. cardiopetalus* Hook.f. & Thomas is an evergreen medium sized tree growing in Palaruvi forests in Kerala, India. To the best of our knowledge no reported chemistry or biology are available on this plant. We have investigated the stem bark of the title plant and isolated and characterized a new styryllactone **1** together with known compounds **2** and **3** (Fig. 1).

Results and Discussion

The ethanol extract of the stem bark was partitioned between hexane/H_2O and EtOAc/H_2O to get two main fractions. The concentrated hexane fraction was chromatographed over a silica gel column and eluted with solvents of increasing polarity to obtain compounds **1**–**3**. Compounds **1**–**3** exhibited the NMR spectral properties of styryllactones. Compound **2**, a pale yellow oil and the major constituent in the extract, was identified as altholactone, a C_{13} furano-pyrone compound initially isolated from an unknown *Polialthea*[11] species and later from *G. giganteus*[3] on the basis of spectral data. Compound **3**, a C_{13} styryllactone, showed a different ^{13}C NMR chemical shift pattern from **2** and was identified as goniopypyrone, the most potent cytotoxic pyrano-pyrone isolated from *G. gigantius*.[6]

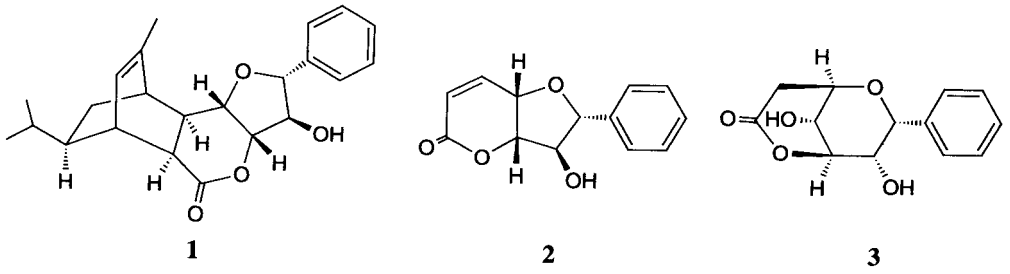

Figure 1. Structures of styryllactones.

Keywords: natural products; styryllactones; structure elucidation; NMR.
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0040–4020/00/$ - see front matter
PII: S0040-4020(00)00948-0

Table 1. 1D and 2D NMR Experiments (400 MHz, d_6-acetone) of **1**

Atom no.	Multiplicity	δC	δH (multiplicity, *J* in Hz)	HMBC correlations
1	C	172.34	–	H-2, H-3
2	CH	42.79	2.76 (dd, 9.8, 2.7); H-2	H-3, H-4^1
3	CH	39.04	2.62 (brd, 9.8); H-3	H-2, H-3^1
4	CH	77.41	4.13 (m); H-4	H-3, H-5, H-6
5	CH	84.36	4.23 (d, 2.0); H-5	H-3, H-4, H-6, H-7
6	CH	84.79	4.10 (d, 2.7); H-6	H-5, H-7
7	CH	88.42	4.64 (d, 2.7); H-7	H-5, H-6, H-9, H-13
8	C	141.18	–	H-6, H-7
9,13	CH	126.91	7.22-7.29 (m); H-9, H-13	H-7, H-10, H-11, H-12
10,12	CH	128.99	7.22-7.29 (m); H-10, H-12	H-9, H-11, H-13
11	CH	128.20	7.22-7.29 (m); H-11	H-9, H-10, H-12, H-13
1^1	C	143.49	–	H-3, H-3^1, H-7^1
2^1	CH	123.39	5.76 (br d, 6.3); H-2^1	H-2, H-3^1, H-4^1, H-7^1
3^1	CH	39.98	2.99 (ddd, 9.8, 2.7, 2.0); H-3^1	H-2, H-4^1, H-5^{1}a
4^1	CH	46.25	1.49 (tdd, 9.2, 5.6, 2.0); H-4^1	H-2, H-5^{1}a, H-8^1, H-9^1, H-10^1
5^1	CH_2	33.24	1.91 (ddd, 12.7, 9.2, 3.3); H-5^{1}a 0.96 (ddd, 12.7, 5.6, 2.8); H-5^{1}b	H-3, H-3^1
6^1	CH	40.96	2.56 (m); H-6^1	H-2, H-2^1, H-3, H-7^1
7^1	CH_3	21.32	1.87 (d, 1.7); H-7^1	H-2^1, H-6^1
8^1	CH	34.02	1.09 (m); H-8^1	H-9^1, H-10^1
9^1	CH_3	20.49	0.80 (d, 6.6); H-9^1	H-8^1, H-10^1
10^1	CH_3	21.32	0.92 (d, 6.6); H-10^1	H-8^1, H-9^1

Compound **1**, isolated as white crystals, was given a trivial name cardiopetalolactone. Its high resolution EI mass spectrum exhibited an M^+ ion at *m/z* 368.2015 corresponding to the formula $C_{23}H_{28}O_4$ (calcd 368.1988). The IR spectrum exhibited bands for a hydroxyl group (3300 cm^{-1}) and a lactone ring (1720 cm^{-1}). The presence of a secondary hydroxyl group was confirmed from the formation of a mono-Mosher ester derivative. The ^1H and ^{13}C NMR spectral pattern of **1** was very different from typical C_{13} styryllactones but partly resembled the signals in furano-pyrone compounds such as altholactone. The five-proton multiplet at δ 7.22–7.29 in the ^1H NMR spectrum indicated a monosubstituted phenyl group. Four deshielded one-proton signals at δ 4.64 (d, *J*=2.7 Hz), 4.23 (d, *J*=2.0 Hz), 4.13 (m) and 4.10 (d, *J*=2.7 Hz) were indicative of oxygen bearing methine protons. An isopropyl group was inferred from two methyl proton doublets at δ 0.80 (d, *J*=6.6 Hz) and 0.92 (d, *J*=6.6 Hz) and a vinyl methyl group from a narrow doublet at δ 1.87 (*J*=1.7 Hz). In addition, the ^1H NMR spectrum exhibited the signal of a vinyl proton at δ 5.76 (br d, *J*=6.3 Hz) indicative of a trisubstituted double bond and several one-proton signals at δ 2.76 (dd), 2.56 (m), 2.62 (br d), 2.99 (ddd), 1.49 (tdd), 1.91 (ddd) and 0.96 (ddd). The $\{^1H\}-^{13}C$ NMR spectrum exhibited the signals of 23 carbon atoms and DEPT experiments showed 20 protonated carbon signals thereby revealing three quaternary carbons in the molecule. The multiplicity assignments of the protonated carbons were made on the basis of DEPT-90 and DEPT-135 experiments. The DEPT-135 spectrum showed 19 positive signals due to CH and CH_3 carbons and one negative signal due to CH_2 carbon. The DEPT-90 sub-spectrum could distinguish sixteen CH carbons among the positive signals thereby indicating three CH_3 carbons in the molecule. The presence of a monosubstituted phenyl ring was evident from the signals at δ 141.18 (one C), 126.91 (two CH), 128.99 (two CH) and 128.20 (one CH). The oxymethine carbon signals at δ 77.41, 84.36, 84.42 and a carbonyl carbon at δ 172.34 were reminiscent of a furano-pyrone moiety with a hydroxyl function. The basic structure of the compound was elucidated by using 2D $^1H-^1H$ COSY, HMQC and HMBC techniques.

The important features of the structure elucidation are the following. The NMR data for compound **1** are given in Table 1. All the direct $^1H-^{13}C$ correlations were traced

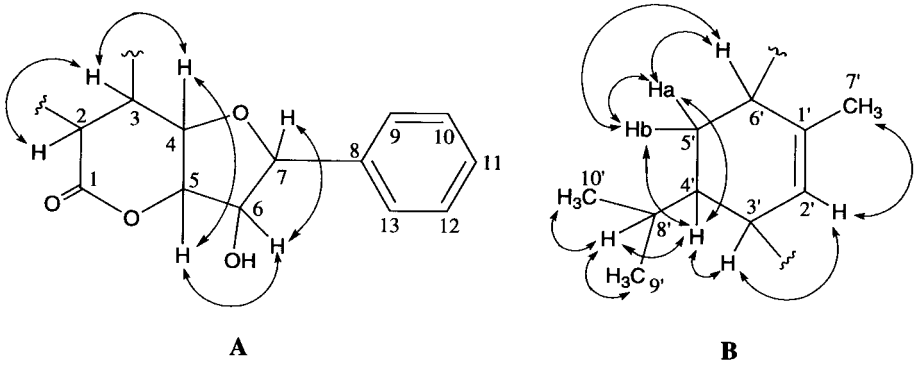

Figure 2. Sub-structures A and B and $^1H-^1H$ coupling networks as revealed by $^1H-^1H$ COSY experiment.

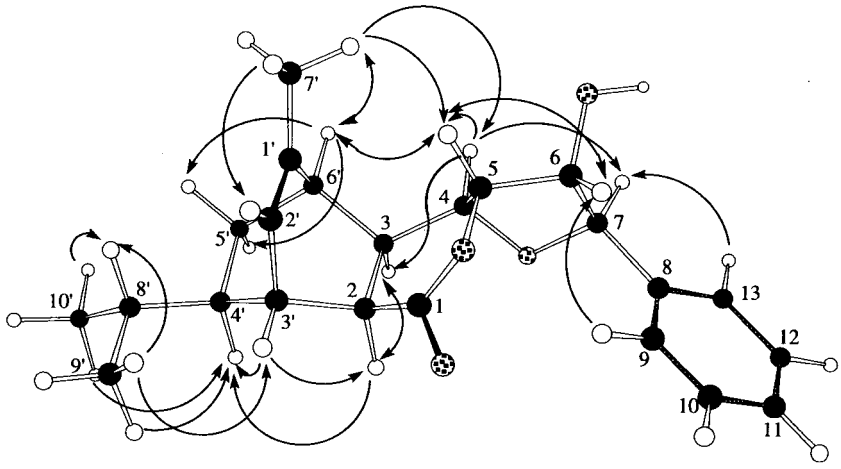

Figure 3. Perspective 3D representation of the structure of **1** showing the relative stereochemistry as revealed by NOE difference experiments.

out from the HMQC spectra. The sub-structure A (Fig. 2) was assigned on the basis of $^1H-^1H$ spin coupling connectivities from the COSY spectrum and also from the long-range $^1H-^{13}C$ correlations in the HMBC spectrum. The second sub-structure B (Fig. 2) was assigned in a similar manner and was found to be a monoterpene unit.

The primary structure of **1** came from a combination of substructures A and B by linking the C-2 carbon of subunit A to the C-3^1 carbon of subunit B and also by linking the C-3 carbon of subunit A to the C-6^1 carbon of subunit B. This combination was based on the additional correlations between H-2 (δ 2.76) and H-3^1 (δ 2.99) and also between H-3 (δ 2.62) and H-6^1 (δ 2.56) observed in the COSY spectrum. In addition to this, the long-range correlations of C-5^1 to H-3, C-4 to H-2 and C-1^1 to H-3 in the HMBC spectrum unambiguously confirmed the primary structure.

The complete relative stereochemistry of the molecule was established by a series of NOE difference experiments and the observed NOE correlations are schematically represented in Fig. 3.

The main features of the NOE correlation studies are the following. The NOE correlation between H-2 and H-3 indicated a *cis* fusion of the lactone and the terpene. The NOE response between H-5 and H-4 established again a *cis* fusion of the tetrahydrofuran and pyrone rings. The NOE response between H-2 and H-4^1 pointed to the position of these protons being on the same side of the molecule, thereby indicating an exo orientation of the isopropyl group as well as the position of the 7^1-Me group on the other side of the molecule as shown in Fig. 3. The irradiation of the 7^1-Me group showed an enhancement in H-4 and H-5 signals indicating that they are on the same side of the molecule. These data, as well as the absence of NOE relationship between H-2 and H-4 or H-5 and H-3, suggested that the two pairs of protons at the ring junctions i.e., H-2, H-3 and H-4, H-5 are on opposite sides of the molecule. The irradiation of H-4 enhanced the H-7 proton signal but did not affect the H-6 signal which suggested a *trans* relationship of phenyl and hydroxyl groups which was further evident from the coupling constant data ($J_{H7/H6}$=2.7 Hz). The NOE correlation of H-8 and H-13 protons with the H-6 and H-7 protons in the tetrahydrofuran ring suggested the preferred conformation of the phenyl group as shown in Fig. 3.

The absolute configuration of **1** was determined by using Mosher ester methodology as follows.[12,13] The absolute stereochemistry of the chiral secondary hydroxyl group at C-6 was determined by preparing the (*S*)- and (*R*)-α-(methoxy)-α-(trifluoromethyl)phenylacetic (MPTA) acid esters of **1** (1*S* and 1*R*) and all the relevant ^1H NMR chemical shifts were assigned unambiguously by $^1H-^1H$ COSY experiments (Table 2). The negative [H-5, $\Delta\delta_{S-R}$=−0.08] and positive [H-7, $\Delta\delta_{S-R}$=+0.08] $\Delta\delta_H$ values were observed for the protons on the left and right segments respectively indicating a 6*R* absolute configuration for **1**. Consequently the absolute configuration of chiral centres in **1** are assigned (2*S*, 3*R*, 4*S*, 5*S*, 6*R*, 7*R*, 3^1R, 4^1R, 6^1R) as depicted in Fig. 3.

The signs of the $\Delta\delta_{S-R}$ for H-7, H-5 and H-4 in **1** were essentially identical with those of etharvensin, a styryllactone with 6*R*, 7*R*, 5*S* and 4*S* absolute configuration.[13] Compound **1** is unique in the category of styryllactones. The relative and absolute stereochemistries at the chiral centres in the lactone part of **1** were found to be identical with those of (+)-altholactone. Therefore it is reasonable to assume that compound **1** is biosynthesized from (+)-althalactone and a monoterpene diene, α-phellandrene, via an enzymatic Diels–Alder reaction. The EI mass spectrum showed retro Diels–Alder ions at *m/z* 136 (100%) and 232

Table 2. Characteristic ^1H NMR (400 MHz, $CDCl_3$) data of Mosher esters **1*S*** and **1*R***

Derivatives	H-9 to H-13	H-7	H-6	H-5	H-4	H-3	H-2
(*S*)-MTPA(1*S*)	7.26–7.37	4.81	5.37	4.33	3.95	3.12	2.85
(*R*)MTPA(1*R*)	7.24–7.34	4.73	5.34	4.41	3.98	3.13	2.86
$\Delta\delta_{S-R}$	+(0.02–0.03)	+0.08	+0.04	−0.08	−0.03	−0.01	−0.01

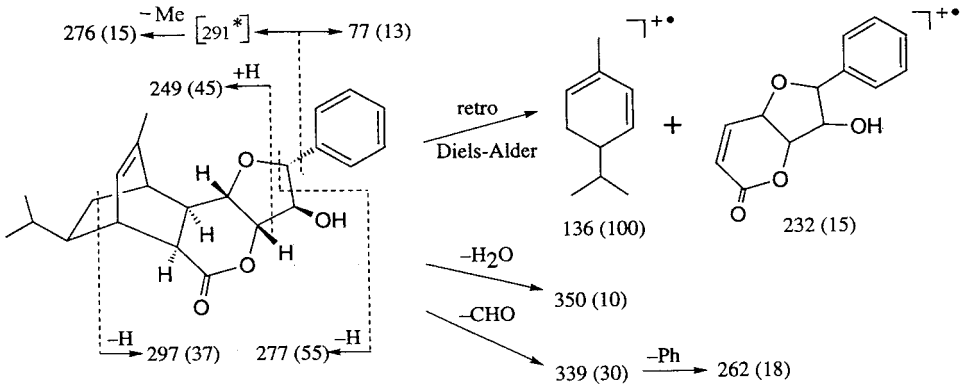

Figure 4. EI Mass spectral fragmentation of **1**. Relative intensities are given in parentheses. * Peak not observed.

(15%) due to monoterpene α-phellandrene and altholactone moieties, respectively (Fig. 4). The rest of the fragment ions were also consistent with the assigned structure for **1**.

Experimental

General

^{1}H and ^{13}C NMR spectra were recorded on JEOL Lamda 400 spectrometer at 24°C. Tetramethylsilane (TMS) was used as the internal standard for ^{1}H NMR and the d_6-acetone signal was used as a reference (δ=29.80) for ^{13}C NMR. All the 1D and 2D acquisitions were accomplished with standard JEOL pulse programs.

HR-EIMS were performed on a JEOL JMS-AX 505HA spectrometer. Thin-layer chromatography (TLC) was performed on Merck F_{254} silica gel plates (0.25 mm thickness) and spots were detected by spraying with vanillin–sulfuric acid followed by heating the plates at 100°C for 5–10 min until the appearance of a spot.

Plant material

Goniothalamus cardiopetalus Hook.f. & Thomas was collected from Palaruvi forests in Kerala, India and was authenticated by Dr Indira Balachandran, Research Officer of the Kottakkal Arya Vaidyasala Herbal Gardens, Kottakkal, Kerala, India where a voucher specimen is deposited.

Extraction and isolation

500 g of shade dried powdered stem bark was repeatedly extracted with ethanol at room temperature. The combined extract (6 l) was concentrated under reduced pressure to get 40 g of brownish viscous material which was first partitioned between hexane/H_2O and later between EtOAc/H_2O to give a hexane extract and an EtOAc extract. The concentrated hexane extract (15 g) was chromatographed over a silica gel column and eluted with hexane, hexane–benzene mixtures, benzene, benzene–dichloromethane mixtures, dichloromethane, mixtures of dichloromethane–EtOAc and finally with ethyl acetate. Stigmasterol (20 mg) was isolated from benzene fractions. Compound **1** (15 mg) was isolated from benzene–dichloromethane fractions. Compounds **2** (500 mg) and **3** (20 mg) were isolated from dichloromethane fractions.

Cardiopetalolactone (1)

White crystals (from acetone). Mp 203°C. IR ν_{max}^{KBr} (cm^{-1}): 3300, 1720. ^{1}H NMR (d_6-acetone): Table 1. ^{13}C NMR (d_6-acetone): Table 1. HR-EIMS: calcd for C_{23} $H_{28}O_4$ (M^+), 368. 1988 found: 368.2015. EI-MS: see Fig. 4.

Preparation of Mosher ester derivatives of 1 (1*S* and 1*R*)

To a solution of 4 mg of **1** in 50 μl of pyridine, 6 μl of (*R*)-α-(methoxy)-α-(trifluoromethyl) phenyl acetyl chloride was added. The reaction mixture was diluted with a small amount of ether and purified by prep.TLC to afford 4 mg of the (*S*)-MTPA ester of **1** (1*S*) as colorless oil. Use of (*S*) α-(methoxy)-α-(trifluoromethyl) phenylacetyl chloride in the same manner with 4 mg of **1** yielded 4 mg of the (*R*)-MTPA ester of **1** (1*R*) as colorless oil.

S-Mosher ester (1*S*)

^{1}H NMR (400 MHz, $CDC1_3$): 0.79 (3H, d, *J*=6.8 Hz, 9^1-Me), 0.89 (3H, d, *J*=6.6 Hz, 10^1-Me), 0.96 (1H, ddd, *J*=12.8, 5.6, 2.9 Hz, H-5^{1}b), 1.08 (1H, m, H-8^1), 1.42 (1H, tdd, *J*=9.2, 5.6, 2.0 Hz, H-4^1), 1.72 (3H, d, *J*=1.4 Hz, 7^1-Me), 1.83 (1H, ddd, *J*=12.6, 9.3, 3.2 Hz, H-5^{1}a), 2.41 (1H, m, H-6^1), 2.63 (1H, br d, *J*=9.8 Hz, H-3^1), 2.85 (1H, dd, *J*=9.8, 2.9 Hz, H-2), 3.12 (1H, ddd, *J*=9.8, 2.7, 2.0 Hz, H-3), 3.53 (3H, s, CH_3 of MTPA), 3.95 (1H, m, H-4), 4.33 (1H, d, *J*=2.2 Hz, H-5), 4.81(1H, d, *J*=2.7 Hz, H-7), 5.37 (1H, d, *J*=2.4 Hz, H-6), 5.80 (1H, d, *J*=6.6 Hz, H-2^1), 7.26–7.37 (5H, m, Ar), 7.43–7.52 (5H, m, Ar of MTPA)

R-Mosher ester (1*R*)

^{1}H NMR (400 MHz, $CDC1_3$): 0.79 (3H, d, *J*=6.8 Hz, 9^1-Me), 0.89 (3H, d, *J*=6.6 Hz, 10^1-Me), 0.97 (1H, ddd, *J*=12.8, 5.6, 2.9 Hz, H-5^{1}b), 1.09 (1H, m, H-8^1), 1.44 (1H, tdd, *J*=9.2, 5.6, 2.0 Hz, H-4^1), 1.76 (3H, d, *J*=2.7 Hz, 7^1-Me), 1.84 (1H, ddd, *J*=12.7, 9.2, 3.2 Hz, H-5^{1}a), 2.45 (1H, m, H-6^1), 2.64 (1H, d, *J*=9.8 Hz, H-3), 2.86 (1H, dd, *J*=9.8, 2.9 Hz, H-2), 3.13 (1H, ddd, *J*=9.8, 2.7, 2.0 Hz, H-3), 3.53 (3H, s, CH_3 of MTPA), 3.98 (1H, m, H-4), 4.41 (1H, d, *J*=2.0 Hz, H-5), 4.73 (1H, d, *J*=2.4 Hz, H-7),

5.34 (1H, d, J=2.4 Hz, H-6), 5.81 (1H, d, J=5.9 Hz, H-2[1]), 7.24–7.34 (5H, m, Ar), 7.43–7.50 (5H, m, Ar of MTPA).

Acknowledgements

The authors thank Mr N. Hara, Department of Chemistry and Materials Science, Tokyo Institute of Technology, for the measurements of EI- and FAB-MS spectra.

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